University of Alberta

The effect of site preparation wounds on aspen quality

by

Jane M. Pankuch

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The effect of site preparation wounds on aspen quality submitted by Jane Margaret Pankuch in partial fulfillment of the requirements for the degree of Master of Science in Forest Biology and Management.

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Abstract

Site preparation wounds may increase stain and decay in the parent roots of aspen [*Populus tremuloides* Michx.]. The study objectives were to determine: (i) if wound type (scrapes versus severs) was related to tree location (furrow versus interfurrow); (ii) if *Armillaria sinapina* Bérubé & Dessureault and *Armillaria ostoyae* (Romagn.) Herink exploit wounds; (iii) if the probability of a wound being infected by *Armillaria* increases with wound size; (iv) the proportion of compartmentalized *Armillaria* infections; and (v) if other pathogenic fungi colonize wounds. A wound survey was conducted in six cut blocks site prepared 8 to 10 years earlier. *Armillaria sinapina* and *A. ostoyae* were determined to be wound pathogens of aspen; however, *A. sinapina* was more frequently associated with wounds than *A. ostoyae*. Sixty percent of *A. sinapina* infected wounds were non-compartmentalized. Sever wounds were associated with furrows; scrape wounds were located both along and between furrows. Pathogenic fungi other than *Armillaria* were not associated with root wounds.

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Dedication

I would like to dedicate this thesis to my parents, Tony and Kathy Pankuch, who always encouraged me to pursue my own interests, whether academic or adventure.

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Chapter 1

General Introduction

1.1 Introduction

The boreal mixedwood forest of Canada extends from southwestern Manitoba to northeastern British Columbia (Rowe 1972). Aspen [*Populus tremuloides* Michx.] and white spruce [*Picea glauca* (Moench) A. Voss] mixtures are common throughout the region; however, balsam poplar [*Populus balsamifera* L.], white birch [*Betula papyrifera* Marsh.], black spruce [*Picea mariana* (Mill.) B.S.P.], balsam fir [*Abies balsamea* (L.) Mill.] and pines [*Pinus* spp.] may also be intermixed (Lieffers *et al.* 1996). In Alberta, the boreal mixedwood region covers approximately 286 000 km^2, which is 43.2% of the land area (Drew 1988).

Historically, aspen was viewed as a nuisance due to its prolific and vigorous sucker regeneration, its widespread occurrence, and its fast growth rate in mixedwood stands (Basham 1993). Management in mixedwood stands involved either the removal of larger diameter conifers, leaving the aspen or clearcutting followed by the use of herbicides to eliminate the aspen (Morley 1986; Lieffers and Beck 1994). The development of oriented strand board and improvements in pulping technology resulted in an increase in the harvesting of hardwood tree species (Lieffers and Beck 1994); forest managers are now aware of an underutilized hardwood fiber resource (Steneker 1976; Morley 1986).

1.2 Aspen Ecology

Aspen is an intolerant pioneer species (Morley 1986) that reproduces primarily by means of root suckering (Day 1944; Farmer 1962; Shier 1973; Steneker 1976; Peterson and Peterson 1992). Suckers arise from lateral roots that have diameters between 0.5 and 2.5 cm (Peterson and Peterson 1992). A secondary root system, which supplements the

primary lateral roots that propagated the clone, develops as the stand ages (Stong and La Roi 1983). Stump and root collar sprouting may also occur if the harvested trees are young (Peterson and Peterson 1992).

Although aspen is a prolific seed producer (Peterson and Peterson 1992), it rarely regenerates from seed, as the seed quickly loses viability after maturing (Steneker 1976; Peterson and Peterson 1992). Seed production per tree varies with age, vigor and site quality (Barnes 1966); seedling establishment requires moist soil conditions, low evaportranspiration, and minimal competition from other ground vegetation (Barnes 1966; Peterson and Peterson 1992).

The disruption of apical dominance and increased soil temperature are the two most important factors involved in the initiation of aspen suckering (Peterson and Peterson 1992). Suckering is inhibited by apical dominance, as a result of auxin being translocated to the roots from the above ground portions of the tree (Peterson and Peterson 1995). Stand replacing disturbances, such as cutting or burning, stimulate suckering by increasing soil temperatures through the exposure of the soil surface (Steneker 1976; Shier and Campbell 1978) and eliminating the flow of auxin into the root system (Peterson and Peterson 1995). The optimal temperature range for suckering is 20° to 30°C (Steneker 1976). The extent of suckering is dependent on the intensity of a stand disturbance, especially the number of trees removed or burned and the amount of ground vegetation removed (Steneker 1976; Maini and Horton 1966a).

Aspen suckers are heavily dependent on the parent root system for the first 25 years following suckering (Zahner and DeByle 1965). DesRochers and Lieffers (2001a) observed living roots on trees which had been dead for several years; these roots were

connected to the roots of live trees by root grafts, or by the original parent root connection. Suckers depend on the parent root system for water and nutrient uptake until secondary root development occurs (DesRochers and Lieffers 2001b); however, live parent roots have been observed in mature aspen trees (Strong and LaRoi 1983).

Aspen is highly susceptible to decay; the high occurrence of heartrot in aspen limits utilization of this tree species (Thomas *et al.* 1960; Basham 1958). In the mixedwood forest of the Boreal Forest Region in Alberta, 73% of the aspen were decayed with the incidence of infection decreasing from mesic sites to dry sites (Thomas *et al.* 1960). There is a weak relationship between aspen decay and age (Basham 1958; Hiratsuka *et al.* 1990); heartrot increases with increasing tree age, as the number of wounds and the time they have been susceptible to infection increases (Basham 1958). Variation in trunk rot within an aspen stand may be partly due to genetic differences among clones (Wall 1971). The most common cause of trunk rot in Alberta is *Phellinus tremulae* (Bondartsev) Bondartsev & Borisov (=*P. igniarius* [Linnaeus: Frie] Quélet, *Fomes igniarius* [Linneaus: Fries] J. Kickx fil. F. *tremulae* Bondartsev). *Peniophora polygonia* (Persoon: Fries) Boudier & Galzin (=*Corticium polygonium* (Persoon: Fries), *Cryptochaete polygonia* (Persoon: Fries) K. Karsten) is the second most prevalent cause of decay in aspen (Hiratsuka *et al.* 1990). *Armillaria* (*Armillaria ostoyae* [Romagn.] Herink and *Armillaria sinapina* [Bérubé and Dessureault]) is the leading cause of aspen butt rot in Alberta (Thomas *et al.* 1960; Hiratsuka *et al.* 1990; Peterson and Peterson 1992). Other common root and butt rot fungi are *Ganoderma applanatum* (Persoon) Patouillard, *Fomitopsis pinicola* (Swartz: Fries) P. Karsten (=*Fomes pinicola* (Swartz:

Fries) Cooke), and *Gymnopilus spectabilis* (Fries: Fries) A.H. Smith (=*Pholiota spectabilis* (Fries: Fries) Gillet) (Hiratsuka *et al.* 1990).

Stain is commonly associated with aspen suckers (Basham 1993); stain or discoloration is caused by various groups of fungi (yeasts, Ascomycotina, and Deuteromycotina) and bacteria (Hiratsuka *et al.* 1990). Blue stain fungi belonging to the genera *Ceratocystis* and *Verticicladiella* are the most likely cause of the "mineral stains" of sapwood in stored logs (Hiratsuka *et al.* 1990). However, sapwood stain may also develop in the absence of microorganisms (Hiratsuka *et al.* 1990).

1.3 Scarification

Aspen stands and mixedwood stands with a high percentage of aspen, are often scarified and subsequently planted with conifer seedlings (Basham 1982); the objective is to improve conditions for the establishment and growth of planted conifer seedlings (Basham 1993). However, aspen sucker regeneration may increase following scarification (Maini and Horton 1966b; Weingartner 1980; Fraser *et al.* 2002). Abundant suckering was observed on heavily scarified or burned sites regardless of whether or not the aspen canopy was removed (Maini and Hortion 1966a). Weingartner (1980) found spring scarification applied prior to suckering resulted in increased sucker density.

Heavy machinery required for scarification causes both stem and root wounds in the surviving suckers (Basham 1982). Stem damage to surviving aspen results from scrapes and pressure wounding on the stem due to scarification machinery that either removes the bark and possibly the outer xylem, or leaves the bark intact and kills the cambium and adjacent phloem (Basham 1988). Suckers in scarified areas tend to have more stem and root decay than suckers in unscarified areas (Basham 1988); variation in

the frequency of infected wounds may be attributed to differences in the size and age of wounds, the position of wounds on the tree and the season of injury (Vasiliauskas 2001). Forestry operations conducted in the winter tend to result in less severe damage to roots and stems (Vasiliauskas 2001).

Basham (1988) observed that aspen suckers surviving in scarified areas had significantly lower average height and stem volume than those in unscarified areas of the same age. Weingartner (1980) found that all scarification treatments resulted in mean dominant heights that were less than those of the controls, irrespective of the timing or intensity of treatment.

1.4 Wounding

1.4.1 Tree Response to Wounding

The majority of pathogens are unable to directly penetrate the corky suberized tissues of the outer bark and rhytidome (Biggs 1992). The outer layers represent preformed anatomical barriers to pathogen ingress (Biggs 1992). Following injury (wounding), a series of predictable and coordinated events occurs, which concludes in the bark of woody plants with the formation of a primary ligno-suberized boundary layer, cell division leading to the formation of wound periderm, the production of callus tissue, new vascular cambium, and eventual wound closure (Biggs 1992). The rapid production of high levels of suberin may determine resistance against fungal pathogens; suberization may be triggered by wounding (Biggs 1992). A chemical signal generated soon after a wound is inflicted may initiate the process resulting in the deposition of suberin (Biggs 1992).

Periderm formation and callus production are both associated with cell division. High levels of auxin promote ethylene production, which indirectly results in periderm formation (Biggs 1992). Wound periderms may be referred to as necrophylactic periderms (Mullick and Jensen 1973). Necrophylactic periderms form whenever dead tissues occur and regeneration of new periderm for continuing protection is necessary; their primary function appears to be the protection of living tissues from the adverse effects associated with cell death (Mullick and Jensen 1973).

Following wounding, the xylem extant at the time of wounding becomes discoloured (Blanchette 1992). The anatomical changes associated with injury-induced discolouration occur within a few weeks. The xylem cells of discoloured wood develop occlusions that fill cell lumina (Blanchette 1992). Discoloured wood tends to have a higher pH and higher moisture and mineral content. Phenols also increase in discoloured wood and the altered wood becomes resistant to colonization by many decay causing Basidiomycetes (Rayner 1986; Blanchette 1992). Tyloses form in vessels filled with air and may slow the process of colonization (Rayner 1986; Blanchette 1992). However, many fungi have the ability to grow through these structures (Blanchette 1992).

The degeneration of cell nuclei and cytoplasm correspond to the production of pigmented substances (Blanchette 1992). Wound size and depth of the wound within the xylem determine the amount and intensity of discoloured wood. The interaction of xylem tissues with air appears to be a requirement for discolouration to occur (Blanchette 1992). Bacteria and fungi may intensify the formation of cell occlusions, resulting in increased discolouration (Blanchette 1992).

1.4.2 Wounds as Infection Courts

Wounds provide important infection courts for many decay causing fungi and provide a means through which fungi may bypass the defense mechanisms of the bark and the sapwood (Woodward 1992). Wounds become increasingly less susceptible to infection with age; damage by wound pathogens will be greatest if infection occurs immediately following wounding (Biggs 1992). Resistance increases as non-specific plant responses to wounding result in the formation of primary ligno-suberized tissues and secondary wound periderm (Biggs 1992).

1.4.3 Limitation of Stain and Decay Associated with Wounds

1.4.3.1 Compartmentalization

According to Shigo (1986), compartmentalization is a dynamic tree defense process that forms boundaries that resist the spread of pathogens. The theory of compartmentalization of decay in trees (CODIT) attempts to explain tree response to injury (Armstrong *et al.* 1981; Rayner and Boddy 1988). The theory attributes the limitation of fungal colonization associated with wounds to an active response in the living sapwood, which directly walls off the sapwood from fungal invasion (Boddy and Rayner 1983; Rayner and Boddy 1988).

The model for compartmentalization describes four walls, which are barriers to infection (Shigo and Tippett 1981); compartmentalization is a two part process (Shigo 1984). Part 1 is represented by three walls (Shigo 1984), which are comprised of tissues extant at the time of injury (Shortle 1979; Shigo 1984). Wall 1 limits vertical spread (Shortle 1979; Shigo and Tippett 1981; Shigo 1984) and consists of occlusions (gums and tyloses) of axial elements (Boddy and Rayner 1983; Rayner and Boddy 1988). Wall 2

resists inward (radial) spread (Shigo and Tippett 1981; Shigo 1984); it is formed by the dense latewood present in each growth ring (Boddy and Rayner 1983; Rayner and Boddy 1988). Wall 3 resists lateral (tangential) spread (Shigo and Tippett 1981; Shigo 1984); it is comprised of the medullary ray tissues (Boddy and Rayner 1983; Rayner and Boddy 1988). Part 2 involves the formation of a barrier zone produced by the cambium (Shigo 1984) and is represented by wall 4, which separates the xylem present at the time of wounding from the xylem formed subsequent to wounding (Shigo and Tippett 1981). Walls 1, 2 and 3 exist prior to wounding and may give way to the spread of microorganisms, while wall 4 forms after wounding and is less likely to fail (Shigo and Tippett 1981). Discoloured and decayed wood may increase in volume within the boundaries of wall 4 (Shigo and Tippett 1981).

1.4.3.2 Theory of Succession

According to the theory of fungal succession, wood is colonized by a variety of different organisms, in succession, following wounding (Shigo 1967; Shigo and Shortle 1979; Rayner and Boddy 1988); this theory is strongly associated with the CODIT concept (Boddy and Rayner 1983). A combination of decay and non-decay causing fungi and other microorganisms inhibit the ability of the tree to compartmentalize infection (Rayner and Boddy 1988). Discolourations initiated by abiotic factors form shortly after wounding, and are enhanced by organisms colonizing the wounds (Shigo 1967). Shigo (1967) found that bacteria and non-hymenomycetous fungi preceded the hymenomycetes. Hymenomycetes are typically found growing through tissues already colonized by other organisms; decayed tissues were surrounded by discoloured tissues yielding bacteria and non-hymenomycetous fungi (Shigo 1967). Bacteria and non-hymenomycetous fungi are

believed to be involved in the detoxification of phenolic substances produced by the trees as a response to injury and infection (Rayner and Boddy 1988).

1.4.3.3 Water and Aeration

As an alternative to the CODIT model, the relative accessibility of woody tissue to permeation by fluids and fungal hyphae may explain the shape of decay and discolouration columns in trees (Boddy and Rayner 1983). When a wound extends deeper than the vascular cambium, the xylem is brought into direct contact with relatively dry air (Rayner 1986). Wounding may create an opportunity for fungi that are unadapted to growth under the microenvironmental conditions present in the intact tree, to colonize woody tissue (Rayner and Boddy 1988). Non-specific mechanisms may be responsible for maintaining sapwood function adjacent to damaged or non-functional tissue (Boddy and Rayner 1983). Air-accessed tissues eventually become sealed off from the rest of the xylem by the formation of tyloses and/or deposition of gums, resins or suberised layers and by the accumulation of polyphenolic extractives (Rayner 1986). These sealant layers have commonly been interpreted as defensive barriers that are produced in direct response to invasion by microorganisms (Rayner 1986). Barrier and reaction zones indirectly contain the spread of fungi as they are formed at sealant layers that limit the spread of cavitation and drying; they mark the boundary between microenvironmental conditions inhospitable to fungi and more favourable conditions in aerated tissue (Rayner 1986; Rayner and Boddy 1988).

1.5 Armillaria Root Disease

1.5.1 Signs and Symptoms

Tree mortality resulting from Armillaria root disease may occur singly or in disease centres, which are openings in a stand (Mallett 1992); mortality in disease centres occurs over many years (Morrison 1981). According to Mallett (1992), the symptoms of *Armillaria* vary with the host and age of the tree. Symptoms typically include foliar discoloration, reduction of growth, stress crops of cones (in conifers), resinosis or gummosis of the lower stem, root rot and butt rot (Morrison 1981; Mallett 1992). There may also be considerably less foliage on infected trees (Mallett 1992).

Signs of the disease include white mycelial fans, rhizomorphs and basidiocarps (Morrison *et al.* 1991; Mallett 1992). Mycelial fans develop between the bark and the wood in the root collar (Morrison 1981; Mallett 1992). In late August or early September *Armillaria* basidiocarps, which are gilled mushrooms, are found at the base of dead or dying trees and stumps in the Canadian prairie provinces (Mallett 1992).

1.5.2 The Infection Process

According to Morrison (1981), the life cycle of *Armillaria* has a parasitic phase, during which new food bases become colonized, and a saprophytic phase, in which the fungus utilizes food bases for growth. *Armillaria* species survive saprophytically in woody substrates in the soil (Redfern and Filip 1991); following the harvesting of wood the fungus colonizes stumps and roots from infections present on roots prior to harvest (Wargo and Shaw 1985). In the next generation, *Armillaria* spreads either by rhizomorphs produced from the roots of infected trees, or by root contact with infested wood material (Wargo and Shaw 1985). Klein-Gebbinck *et al.* (1991) determined that

stumps, roots and debris from the previous generation were the inoculum source in 78% of infected juvenile trees, while infected regeneration served as the inoculum source for the remaining trees.

Thomas (1934) defined infection by *Armillaria* as penetration of the fungus into the host with or without further development. Stump infection may result following fusion of two haploid mycelia each derived from a basidiospore (Rishbeth 1985); however, basidiospores are not thought to play an important role in the dispersion of the pathogen (Redfern and Filip 1991; Mallett 1992). Other substrates may be colonized by the extension of rhizomorphs through the soil (Rishbeth 1985; Wargo and Shaw 1985; Redfern and Filip 1991; Mallett 1992). Infested stumps or dead trees provide a food base for rhizomorph extension (Mallett 1992). Direct mycelial transfer may also occur through root-to-root contact or by contact with infested woody material in the soil (Rishbeth 1985; Wargo and Shaw 1985; Mallett 1992).

1.5.2.1 Basidiospores

Basidiospore production provides a source of genetic diversity and facilitates long-range spread (Redfern and Filip 1991). It is unlikely that basidiospores can directly infect large woody roots, since spores have little energy reserves and thus low inoculum potential (Redfern and Filip 1991); basidiospores are only thought to infest dead stumps (Campbell 1934). Rishbeth (1978) observed *A. mellea* foci in association with oak and beech thinning stumps that had been infested by basidiospores. Dettman and van der Kamp (2001) inferred that the new infection of available substrates via airborne basidiospores occurs more frequently with *A. sinapina* than with *A. ostoyae*; dispersion

via basidiospores may play a more important role in the epidemiology of *A. sinapina* than previously thought.

1.5.2.2 Rhizomorphs

Infections are more commonly initiated by rhizomorphs than root contacts (Klein-Gebbinck *et al.* 1991). Rhizomorphs are a collection of specialized hyphae surrounded by a black protective rind (Manion 1991); rhizomorphs of *Armillaria* may grow out in all directions from infested wood in the soil (Garrett 1956). Primary sources of rhizomorphs include roots, debris, and stumps from the previous stand (Klein-Gebbinck *et al.* 1991). In Alberta, the roots of infected trees in the current stand (roots of juvenile trees) are secondary sources of rhizomorph production, and initiate fewer infections than those from trees in the previous generation (Klein-Gebbinck *et al.* 1991).

Rhizomorphs become attached to roots as the mucilaginous substance which envelops the growing tip of rhizomorphs hardens (Thomas 1934). The outer layer of cork cells can be penetrated by single side hyphae that develop from the rhizomorph tip and anchor the rhizomorph to the root. Branch rhizomorphs develop at the point of contact between the rhizomorph and the root surface. Branches penetrate as a unit by a combination of mechanical and chemical means (Thomas 1934); large amounts of protein and nucleic acids are present in the apical region of the rhizomorph (Motta 1971). Further advance of rhizomorphs is preceded by cell death (Thomas 1934). Following the penetration of a susceptible host, rhizomorphs grow rapidly, causing destruction of host tissue (Thomas 1934). Penetration of host tissue occurs in roots of resistant hosts; however, the fungus is unable to establish itself and cause any decay (Thomas 1934). Lesions on large roots resulting from infection by *A. ostoyae* are often confined to bark

tissues by the formation of necrophylactic periderms (Robinson and Morrison 2001). Necrophylactic periderms with multiple bands of phellem provide additional resistance to the spread of *A. ostoyae* infection (Robinson and Morrison 2001).

1.5.3 Decay

Armillaria root disease results in a white rot of woody tissues, as both lignin and cellulose are decomposed (Morrison *et al.* 1991). Tissues in the advanced stages of decay become soft and wet (Campbell 1934). Wood decayed by *Armillaria* typically contains pseudosclerotial plates, which appear in cross section as zone lines (Campbell 1934; Lopez-Real 1975). Pseudosclerotial plate formation requires that free water be present in the lumina of the wood cells; the pattern of plate formation corresponds to the moisture content of wood (Lopez-Real and Swift 1975). In the early stages of decay these plates are light brown in colour and become black in older rots (Campbell 1934). Pseudosclerotial plates are comprised of pigmented bladder hyphae, similar to the hyphae comprising the outer rind of mature rhizomorphs (Campbell 1934; Lopez-Real 1975; Morrison *et al.* 1991).

1.5.4 Biological Species

Armillaria root disease is an important disease of both deciduous and coniferous tree species throughout the world (Wargo and Shaw 1985). The disease was once thought to be caused by the species *Armillaria mellea* (Vahl:Fries) Kummer (Mallett 1992). In North America there are several species of *Armillaria* (Dumas 1988; Mallett 1992), which have been termed North American biological species (NABS) (Mallett 1992). Anderson and Ullrich (1979) identified ten intersterile groups of *A. mellea* in

North America. Anderson (1986) revised the number of NABS from ten to eight; Morrison *et al.* (1985) found an additional group, designated NABS XI. The identity of the nine NABS of *Armillaria* are listed in Table 1-1.

Table 1-1. Identity of the North American Biological Species (NABS) of *Armillaria* (Mallett 1992; Banik *et al.* 1996; Volk *et al.* 1996)

NABS	Species Name
I	*Armillaria ostoyae* (Romagn.) Herink
II	*A. gemina* Bérubé & Dessureault
III	*A. calvescens* Bérubé & Dessureault
V	*A. sinapina* Bérubé & Dessureault
VI	*A. mellea* (Vahl.:Fries) Kummer
VII	*A. gallica* Marxmüller & Romagn.
IX	*A. nabsnona* Volk & Burdsall
X	taxomically undescribed
XI	*A. cepistipes* Velenovsky

1.5.5 Geographic Distribution

According to Anderson and Ullrich (1979), distinguishing between biological species based on geographic distribution is difficult; several of the groups are broadly distributed in North America. In North America, *A. ostoyae* has a very wide distribution, which extends from the Pacific to the Atlantic coast; however, it is predominantly located in the northern temperature region (Bérubé and Dessureault 1988). *Armillaria ostoyae*, *A. sinapina*, *A. gallica*, *A. nabsnona*, *A. cepistipes* and NABS X have been found in British Columbia (Morrison *et al.* 1985). Four intersterile species were found in the

mixedwood forest of northern Ontario and include: *A. ostoyae*, *A. sinapina*, *A. gallica* and *A. calvescens* (Dumas 1988). McLaughlin (2001) reported six *Armillaria* species in central and southern Ontario: *A. gallica*, *A. calvescens*, *A. ostoyae*, *A. sinapina*, *A. gemina* and *A. mellea*. In Quebec, *A. ostoyae*, *A. gemina*, *A. calvescens*, *A. sinapina*, and *A. mellea* have been recorded (Bérubé and Dessureault 1988, 1989). In Manitoba and Saskatchewan, *A. ostoyae*, *A. sinapina* and *A. calvescens* were found (Mallett 1990).

1.5.6 Host and Site Association

Conifers are the preferred host of *A. ostoyae*; however, broadleaved trees may also be attacked (Blodgett and Worrall 1992; Morrison *et al.* 1985; Dumas 1988; Morrison and Mallett 1996; McLaughlin 2001). Klein-Gebbinck *et al.* (1991) reported infections caused by *A. ostoyae* on fireweed (*Epilobium angustifolium* L.), grass plants, rose (*Rosa* sp.), bearberry (*Artostaphylos uva-ursi* (L.) Spreng.) and willow (*Salix* sp.). In Ontario, *A. ostoyae* typically occurs on dry to fresh, rapidly drained sandy to coarse loamy sites; it was absent from sites with finer textured soils (McLaughlin 2001).

Armillaria sinapina is typically associated with deciduous tree species (Dumas 1988; Mallett 1990; Blodgett and Worrall 1992). In the prairie provinces, Mallett (1990) rarely isolated *A. sinapina* from conifer hosts. *Armillaria sinapina* is commonly associated with noncalcareous sites in Ontario and was found in all moisture regimes; however, it was more frequently found on poorly drained sites (McLaughlin 2001).

1.5.7 Pathogenicity

Pathogenicity among *A. ostoyae* in North America is quite variable; isolates were usually saprophytes, but occasionally were mild pathogens on declining trees in Quebec

(Bérubé and Dessureault 1988). However, *A. ostoyae* was determined to be moderately to highly pathogenic on conifers in British Columbia (Morrison *et al.* 1985; Dettman and van der Kamp 2001); when both *A. ostoyae* and *A. sinapina* were present in the same infection centre, *A. ostoyae* was always the dominant species (Dettman and van der Kamp 2001). Once established, *A. ostoyae* is able to spread quite aggressively and capture significant amounts of secondary resources, which involves new infection of available subtrates via rhizomorph growth (Dettman and van der Kamp 2001). Blodgett and Worrall (1992) reported *A. ostoyae* causing infection in the live cambium on a greater proportion of its hosts than other *Armillaria* species. In Wisconsin and adjacent areas, *A. ostoyae* was more frequently isolated from lesions on healthy roots of aspen and from roots of recently killed trees than *A. sinapina*; therefore, it appears to be more pathogenic on aspen than *A. sinapina* (Banik *et al.* 1995).

Armillaria sinapina is weakly pathogenic in British Columbia, as it is usually found on suppressed or overmature broadleaved tree species (Morrison *et al.* 1985). In northern Ontario, *A. sinapina* primarily occurs on dead material (Dumas 1988). Mugala *et al.* (1989) observed that *A. sinapina* was only weakly pathogenic on conifers in Alberta; this species did not cause mortality in conifers. *Armillaria sinapina* depends on an extensive rhizomorph network in soil and on root surfaces to exploit newly available food bases (Cruickshank *et al.* 1997). Sites with conditions that are optimal for rhizomorph growth tend to favour *A. sinapina* over *A. ostoyae*, as *A. ostoyae* produces fewer rhizomorphs (Cruickshank *et al.* 1997).

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Chapter 2

The Effect of Site Preparation Wounds on Aspen Quality

2.1 Introduction

Following logging or natural disturbance, aspen [*Populus tremuloides* Michx.] reproduces vegetatively by suckers that develop from shallow lateral roots (Schier 1973; Peterson and Peterson 1992; DesRochers and Lieffers 2001), also known as parent roots. As the stand ages, a secondary root system develops from the suckers to supplement the parent root system (Strong and La Roi 1983). Site preparation operations performed for the regeneration of white spruce [*Picea glauca* (Moench) Voss] may benefit aspen by increasing soil temperature (Maini and Horton 1966). However, it is possible that injury of parent roots during site preparation could lead to an increase in disease in the regenerating stand. For example, Basham (1988) determined that the root systems and stems of aspen suckers injured by scarification had significantly more stain and decay than uninjured suckers perhaps because of wounding of the parent roots.

Disk trenchers and ripper plows have often been used to create planting sites for spruce. Both techniques produce a series of continuous furrows or trenches evenly spaced throughout the cut block. The interfurrow areas between the trenches are approximately 2.5 metres wide; sites scarified using a ripper plow produce a deeper trench than a disk trencher. A preliminary study of aspen roots from sites scarified for the regeneration of white spruce determined that the disk trencher and ripper plow methods of site preparation produced three types of wounds: scrapes, severs and sever/scrape combinations (a root severed and scraped at the same location) (Pankuch unpublished).

The amounts of stain and decay following injury may depend on the ability of trees to contain microorganisms. The CODIT (Compartmentalization Of Decay In Trees)

model attempts to explain this containment process (Armstrong *et al.* 1981; Rayner and Boddy 1988). According to this model, any stain or decay present in the stem or roots is restricted to the tissues that were formed at the time of wounding (Shigo and Tippett 1981). In the parent roots of aspen the number of growth rings between compartmentalized stain or decay and the cambium present should equal the number of years since site preparation occurred. However, there is evidence that stain and decay resulting from infection by *Armillaria* is not compartmentalized; stain and decay were often observed across the entire cross section of aspen roots, including the most recently produced xylem (Stanosz and Patton 1987). Infection of aspen by *Armillaria* species is already cause for concern as the root and butt rot it causes reduces wood quality and volume (Stanosz and Patton 1987; Basham 1958; Thomas *et al.* 1960). If *Armillaria* is not compartmentalized, the risk of stain and decay from the parent roots to spread into the root collar and the adventitious roots (Basham 1988) would seemlingly be increased.

The overall objective of this study was to determine the effect of site preparation wounds on aspen quality. The specific objectives of this study were to determine: (i) if wound type (scrapes versus severs) was related to tree location (furrow versus interfurrow); (ii) the degree to which *Armillaria sinapina* Bérubé & Dessureault and *Armillaria ostoyae* (Romagn.) Herink require or can exploit wounds as infection courts; (iii) if the probability of a wound being infected by *Armillaria* increases with wound size; (iv) the proportion of *Armillaria* infections that were compartmentalized; and (v) if other pathogenic fungi frequently colonize wounds. If site preparation increases the frequency of *Armillaria* infections and if those infections are not compartmentalized, site reparation could lead to an increase in this common disease of aspen.

2.2 Materials and Methods

2.2.1 Cut Block and Ecological Selection Criteria

A survey of site preparation wounds was conducted in six cut blocks selected from within Weyerhaeuser's Edson and Drayton Valley Forest Management Areas. The cut blocks had been site prepared 8 to 10 years previously, prior to the planting of white spruce seedlings. The choice of cut block age was a compromise; increasing cut block age allows more time for fungal colonization of wounds but makes the identification of scarification wounds more difficult. The average densities of white spruce and aspen in the cut blocks were 1200 and 4000 trees per hectare, respectively. All of the cut blocks were located within the e2 (low-bush cranberry AW) ecosite phase of the lower foothills subregion of Alberta (Beckingham *et al.* 1996). This ecosite phase is characterized by a mesic moisture regime, medium nutrient regime and moderately fine to fine-textured till or glaciolacustrine parent materials.

Site preparation was done with either a ripper plow or disc trencher. An effort was made to select cut blocks from different Township and Range locations; the two cut blocks located in the same Township and Range were 2.2 km apart. Table 2-1 summarizes the location and forestry operation dates for the six cut blocks.

Table 2-1. Cut block location and forestry operation dates.

Cut Block	Location *	Harvest Date	Site Preparation Date	Method of Site Preparation
14	Twp 45 Rge 12	01-Dec-1990	01-Aug-1991	Disk Trencher
26	Twp 44 Rge 12	01-March-1990	01-Aug-1991	Disk Trencher
61	Twp 42 Rge 07	01-Sept-1990	01-Aug-1991	Disk Trencher
80	Twp 48 Rge12	30-Oct-1992	30-June-1993	Disk Trencher
M14	Twp 50 Rge 10	20-Feb-1992	20-March-1992	Ripper Plow
M16	Twp50 Rge 10	09-Jan-1992	20-March-1992	Ripper Plow

*Twp = Township; Rge = Range West of the 5th Meridian

2.2.2 Root Collection

A starting point was arbitrarily located along the roadside edge of each cut block; a transect direction was selected from the start point to provide maximum coverage of the cut block. Sampling of the root systems was systematic; 30 plots spaced approximately 20 to 30 m apart were located along the transect. At the odd numbered plots, the root system of an aspen tree was removed from along a furrow, while at the even numbered plots the root system was removed from an interfurrow area. All roots within 30 cm of the base of the stem were excavated and brought back to the laboratory for further examination.

2.2.3 Wound Classification

In the laboratory, the root systems were washed to remove any loose soil; the parent roots of each root system were identified and diagramed. All wounds on the parent root system were categorized as either scrapes (Figure 2-1), severs (Figure 2-2) or sever/scrape combinations (Figure 2-3).

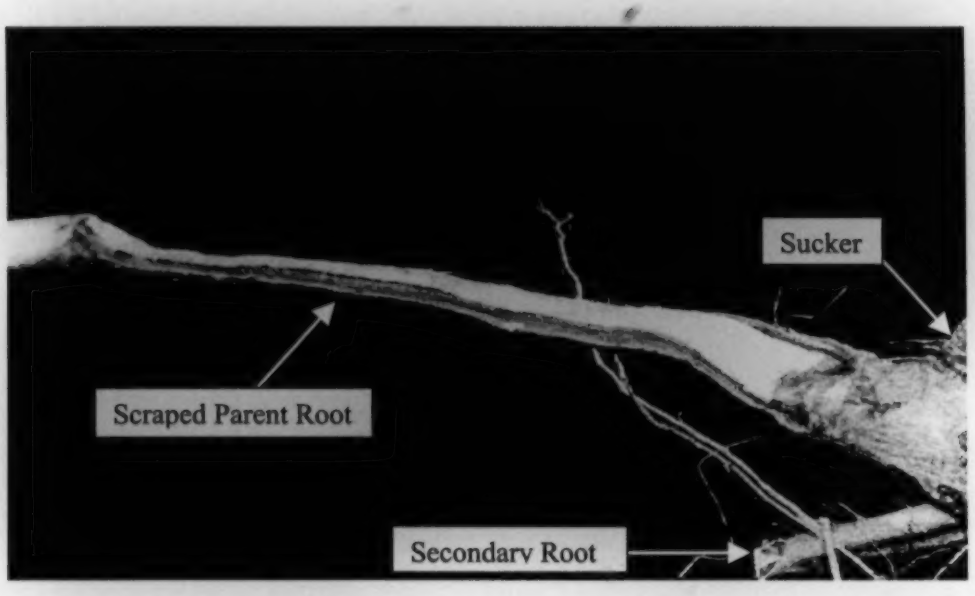

Figure 2-1. Scrape site preparation wound located on an aspen parent root.

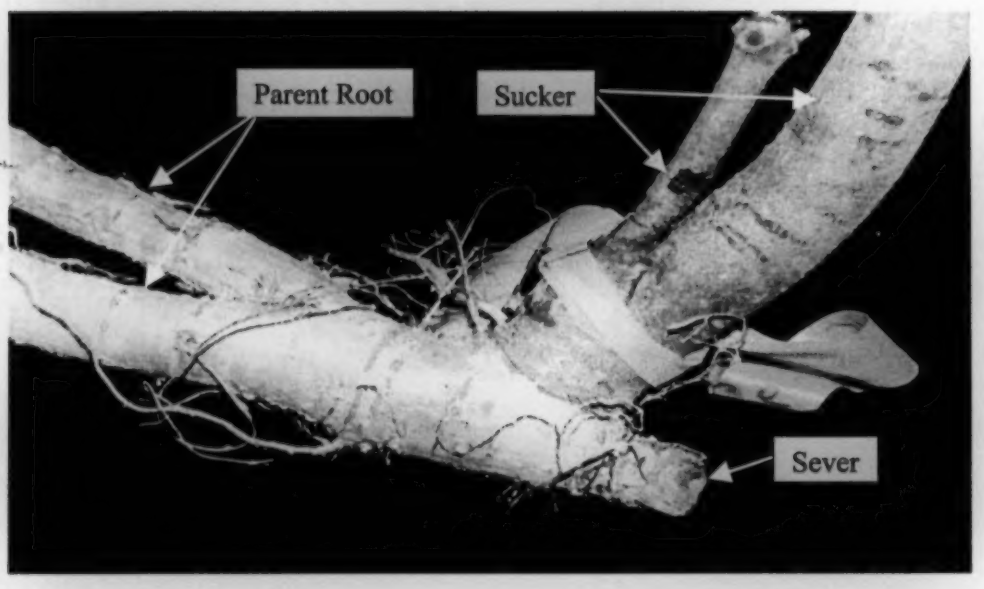

Figure 2-2. Sever site preparation wound located on an aspen parent root.

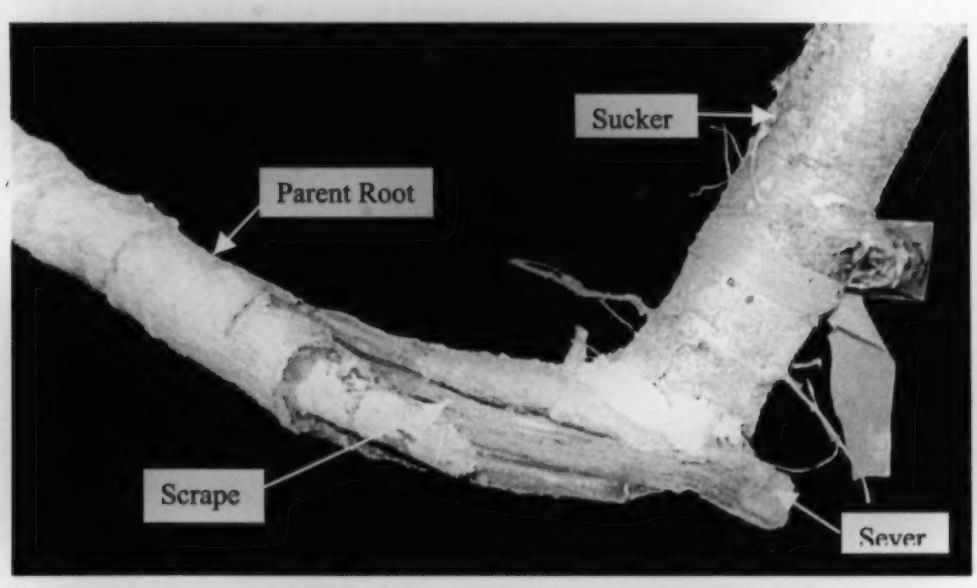

Figure 2-3. Sever/scrape combination site preparation wound located on an aspen parent root.

Root systems in which only one side of the parent root could be identified and which had no associated stain or decay were categorized as presumptive severs. Presumptive severs accounted for only 2 percent of the total number of wounds and were not included in any of the statistical analyses.

2.2.4 Root Surface Area

The parent root surface area of each root system was determined using a modification of the formula for a frustum of a cone: surface area = s((C+c)/2), where s = the length of the side of the frustum; C = the circumference of the large end of the root; and c = the circumference of the small end of the root. The circumference of the large and small ends and the length of each root segment were measured to the nearest tenth of a centimetre. The surface area of all parent root segments per root system was calculated using the above formula. The number of parent root segments per root system was

dependent on the degree of parent root branching and whether or not the parent root had been severed; each root system had from one to four parent root segments. The total parent root surface area of each root system was determined by summing the surface areas of each root segment.

Each wound was measured from inside callus to inside callus and the dimensions recorded to the nearest tenth of a centimetre. Individual wound surface areas were calculated using the formula for either a rectangle (length*width) or a circle ($\pi*r^2$), depending on the wound type. The total wounded surface area of each root system was calculated by summing the surface area measurements of each wound.

Possible *Armillaria* infections on unwounded root tissues were recognized by the presence of swellings and cracks in the bark, or rhizomorphs attached to the outer root surface. Confirmation of infection was accomplished by dissection, isolation and identification as described below.

2.2.5 Isolation of Fungi

Wounded and unwounded parent root tissues were sectioned using a hand saw to determine if they were stained and/or decayed. A thin cross section was cut from each wound and set aside for evaluation of compartmentalization. The cut wood faces were surface sterilized by wiping them with a sterile cloth soaked in a solution containing 80% sterile distilled water, 10% ethanol (95% concentration) and 10% bleach (5% sodium hyperchlorite). Separate isolations were attempted from each differently stained or decayed area for all wounds within 72 hours of collection. A 1-cm^2 piece of wood was removed using a scalpel, to expose the underlying wood. Four 1-mm^2 pieces of wood were aseptically removed from the exposed wood surface. Two of the wood pieces were

placed on malt extract agar (Difco malt extract, 20 g/L; Difco agar, 17 g/L) acidified with 1 mL/L 25% lactic acid post autoclave and amended with 100 mg streptomycin/L, and two were placed on Benomyl-Dichloran-phenol (BDP) agar (1 L potato dextrose agar with 5 mL BDP stock solution (1 g phenol; 0.32 g Benomyl 50% WP; 0.16 g Dichloran; 100 mL 50% ethanol; 2 g streptomycin; 1 g tetracycline)). Isolations from suspected *Armillaria* infections in unwounded root tissues were attempted from both the phloem and xylem. The culture plates were sealed with parafilm and incubated in the dark at room temperature; subculturing was done as necessary.

2.2.6 *Armillaria* Species Identification

Isolates resembling *Armillaria* on either malt extract agar or BDP agar were recognized by a crustose colony morphology and/or the presence of rhizomorphs; these isolates were transferred to carrot agar (300 g carrots; 17 g agar; 800 mL distilled water) and grown at room temperature for 3 weeks. One *Armillaria* isolate from each *Armillaria* infected wound or unwounded root area was randomly selected for species identification. The isolates were identified as either *A. sinapina* or *A. ostoyae* using a polymerase chain reaction (PCR) method and restriction fragment length polymorphisms (RFLP) in the rDNA IGS-1 region (Harrington and Wingfield 1995; White *et al.* 1998; Thormann *et al.* 2001). The amplified DNA of each isolate was digested with the *Alu* I restriction enzyme, electrophoresed in agarose gels, stained with ethidium bromide and visualized using ultraviolet light; the PCR and RFLP methodology used in this study were similar to that described in Thormann *et al.* (2001).

Armillaria ostoyae and *A. sinapina* have more than one *Alu* I restriction fragment pattern (Harrington and Wingfield 1995; White *et al.* 1998). The amplification products

of isolates of *A. ostoyae* and *Armillaria gemina* Bérubé & Dessureault have the same *Alu* I restriction sites for one fragment pattern (Harrington and Wingfield 1995). To rule out the possibility of an isolate being *A. gemina*, isolates yielding the fragment pattern 310, 200, 135 base pairs (bp) were digested with the restriction enzyme *Nde* I; only isolates of *A. ostoyae* were cleaved (Harrington and Wingfield 1995). The amplification products of isolates of *A. sinapina* and *Armillaria gallica* Marxmüller & Romagn. have the same *Alu* I restriction sites for one fragment pattern (White *et al.* 1998). To determine if isolates having the fragment pattern 399, 240, 183 bp were *A. sinapina* or *A. gallica* the IGS-2 region would have to be amplified and digested with the *Alu* I restriction enzyme (White *et al.* 1998). Therefore, any isolates possessing this fragment pattern were assumed to be *A. sinapina*, as *A. gallica* is not known to occur in Alberta.

2.2.7 Non-*Armillaria* Species Identification

Non-*Armillaria* Basidiomycotina fungi, recognized by the presence of clamp connections, were recorded as unknown clamped fungi. The most commonly isolated Deuteromycotina were identified by light microscopy. Slide cultures of these fungi were prepared by first placing a sterile coverslip on a plate of water agar (15 g/L agar). A 0.25 cm^2 block of cereal agar (50 g/L Pablum; 15 g/L agar) was then placed on top of the coverslip and inoculated with a small amount of mycelium from the unknown isolate. A second sterile coverslip was then placed on top of the mycelium. The water agar plates were sealed with parafilm and incubated in the dark at room temperature for 3 to 5 days, following which the top coverslip was carefully removed with sterile tweezers and placed on a slide containing acid fuchsin stain. Deuteromycotina fungi repeatedly isolated from wounds were grouped based on cultural and microscopic characteristics. One

representative of each Deuteromycotina group was sent to the University of Alberta Microfungus Collection and Herbarium (UAMH) for identification; groups selected for identification were isolated from a minimum of 25 different wounds. Any fungi not commonly isolated from aspen were grouped under the heading "unidentified fungi."

2.2.8 Compartmentalization

The cross section removed from each *Armillaria* infected wound was used to determine whether or not compartmentalization had occurred. To improve the visibility of the small growth rings, the surface of each cross section was cut with a razor blade and white chalk was applied to the smooth surface (DesRochers and Lieffers 2001). The number of growth rings between the outermost extent of the stain or decay and the cambium were counted with the aid of a dissecting microscope. If the number of these growth rings was greater than or equal to the number of years since site preparation, the wound was categorized as compartmentalized. If the number of growth rings was less than the number of years since site preparation, the wound was categorized as non-compartmentalized. In 7 percent of the *Armillaria* infected wounds it was difficult to determine an accurate growth ring count; these wounds were categorized as questionable, and were not included in any of the statistical analyses. Only those wounds within 30 cm of the aspen stem were evaluated for compartmentalization, as the growth rings beyond this distance from the stem become difficult to count.

2.2.9 Statistical Analysis

All statistical analyses were performed using the SAS (SAS Institute Inc., Cary, NC, USA) statistical package; significance was assessed using $\alpha = 0.05$. To determine if

site preparation technique affected the frequency and type of wounding, the wound data were analyzed using multivariate analysis of variance (MANOVA), assuming a split plot design. The model was:

$$\underline{Y}_{ijk} = \underline{\mu} + \underline{T}_i + \underline{B}_j(\underline{T}_i) + \underline{L}_k + (\underline{TL})_{ik} + \underline{\varepsilon}_{ijk}$$

$$i = 1,2; \qquad j = 1,2,3,4,5,6; \qquad k = 1,2$$

where:

$\underline{Y}_{ijk}$ = the vector of the % of trees with at least one scrape, the % of trees with at least one sever and the % of unwounded trees. Sever/scrape wounds were considered as both scrapes and severs.

$\underline{\mu}$ = the vector of the overall means;

$\underline{T}i$ = the vector of the fixed effect of the i^{th} site preparation technique (ripper plow or disk trencher);

$\underline{B}_j(\underline{T}_i)$ = the vector of the random effect of the j^{th} cut block nested within the i^{th} site preparation technique; the error term for the site preparation technique effect;

$\underline{L}_k$ = the vector of the fixed effect of the l^{th} location (furrow or interfurrow);

$\underline{TL}_{ik}$ = the vector of the fixed interaction of the i^{th} site preparation technique and the k^{th} location;

$\underline{\varepsilon}_{ijk}$ = the vector of the random effect of the ijk^{th} treatment effect; the error term for location and site preparation technique by location interaction.

Friedman's test (Stokes *et al.* 1995) was then used to determine if the frequency of different wound types varied with the location of trees within or between furrows, while controlling for the effect of cut block. This test provided a more focused test of the association between wound type and tree location. The test was performed on the number of trees having either (i) at least one scrape, (ii) at least one sever or (iii) no wounds. Sever/scrape combination wounds were considered as both scrape and sever wounds.

To compare the incidence of infection of wounded and unwounded tissues by *A. ostoyae* and *A. sinapina* and to evaluate if this occurrence varied with site preparation

technique, the infection data were analyzed as a split plot. The main plot factor was site preparation technique. The subplot factors were wound status (wounded or unwounded) and *Armillaria* species (*A. ostoyae* or *A. sinapina*). The dependent variable was the number of infections/m^2 for each combination of site preparation technique, cut block, wound status and *Armillaria* species. The dependent variable was log transformed after adding a value of 1.0 (one percent of the mean number of infections per m^2) to each observed value. The model was:

$$Y_{ijkl} = \mu + T_i + B_j(T_i) + A_k + S_l + (TA)_{ik} + (TS)_{il} + (AS)_{kl} + (TAS)_{ikl} + \varepsilon_{ijkl}$$

$$i = 1,2; \qquad j = 1,2,3,4,5,6; \qquad k = 1,2; \qquad l = 1,2$$

where:

Y_{ijkl} = the number of infections/m^2;
μ = the overall mean;
Ti = the fixed effect of the i^{th} site preparation technique (ripper plow or disk trencher);
$B_j(T_i)$ = the random effect of the j^{th} cut block nested within the i^{th} site preparation technique; the error term for the site preparation technique effect;
A_k = the fixed effect of the k^{th} *Armillaria* species (*A. ostoyae* or *A. sinapina*);
S_l = the fixed effect of the l^{th} wound status (wounded or unwounded);
TA_{ik} = the fixed interaction of the i^{th} site preparation technique and the k^{th} *Armillaria* species;
TS_{il} = the fixed interaction of the i^{th} site preparation technique and the l^{th} wound status;
AS_{kl} = the fixed interaction of the k^{th} *Armillaria* species and the l^{th} wound status;
TAS_{ikl} = the fixed interaction of the i^{th} site preparation technique, the k^{th} *Armillaria* species and the l^{th} wound status;
ε_{ijkl} = the residual, used as the error term for the subplot factors.

Because error variances differed among treatments, the MIXED procedure in SAS was used to incorporate these heterogenous errors.

Logistic regression was used to determine if infection of wounds by *A. sinapina* was influenced by wound size or type. Wounds were classified as either infected or uninfected by *A. sinapina*, and the resultant binary value was used as the dependent variable in the following model:

$$Y_{ijk} = \mu + B_i + T_j + S_k + (TS)_{jk} + \varepsilon_{ijk}$$

$$i = 1,2,3,4,5,6; \qquad j = 1,2; \qquad k = 1,...n$$

where:

Y_{ijk} = Log odds of ijkth wound being infected by *A. sinapina*;
μ = the overall mean;
B_i = the effect of the ith cut block;
T_j = the effect of the jth type of wound;
S_k = the effect of the kth size of wound;
TS_{jk} = the interaction of the jth type of wound and the kth size of wound;
ε_{ijk} = the residual

An analysis of several logistic regression models containing combinations of the explanatory variables (cut block, wound type, and wound size) was performed to select the most parsimonious model.

Fisher's exact test (Stokes *et al.* 1995) was used to determine if the probability of an *A. sinapina* infected wound being compartmentalized varied with the type of wound. The data were combined across the six cut blocks to increase the number of observations per category. This test was not performed on the *A. ostoyae* data, as there were only 12 *A. ostoyae* infected wounds.

2.3 Results

Only two percent (0.15 m^2) of the total parent root surface area was wounded; 98 percent (6.37 m^2) was unwounded. There were 289 wounds associated with the parent root systems of 146 trees. The parent roots of 28 trees were unwounded; the parent roots of six trees could not be identified and were excluded from all analyses.

Sever wounds were more common along furrows than between them (Figure 2-4). In contrast, unwounded trees were more common between furrows; on average 6 percent of furrow trees and 27 percent of interfurrow trees were unwounded. Approximately 64 percent of the trees had scrape wounds, irrespective of whether they were located alongside or between furrows (Figure 2-4).

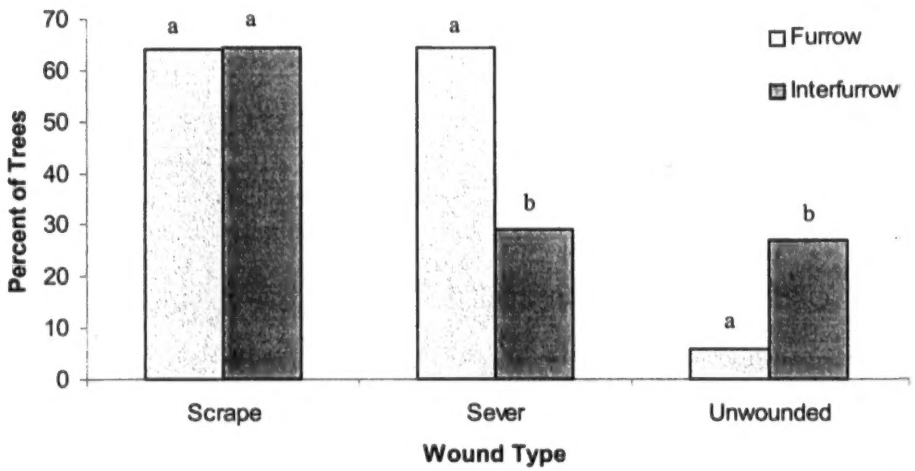

Figure 2-4. Average percentage of trees having either at least one scrape, at least one sever, or no wounds. Trees with sever/scrape combination wounds were considered to have both scrapes and severs. Letters above bars indicate significant differences between furrow and interfurrow trees within wound type ($p<0.05$).

The MANOVA indicated that although tree location (furrow versus interfurrow) affected the relative amounts of scraped, severed and unwounded trees ($p=0.0464$), site

preparation technique (ripper plow versus disk trencher) had no effect on the frequency and type of wounding ($p=0.5950$). Furthermore, the effect of tree location was consistent for the different site preparation techniques as reflected by the lack of a site preparation by location interaction ($p=0.2328$).

Rhizomorphs were observed on nearly all sampled root systems, either epiphytically or in association with *Armillaria* infections of wounded or unwounded roots. Epiphytic rhizomorphs were not necessarily an indication of infection. Root dissection beneath the point of epiphytic attachment indicated that phloem infection typically did not occur. *Armillaria* infections through wounded tissues often had rhizomorphs attached to the exposed xylem surface; sectioning of *Armillaria* infected wounds often revealed the presence of pseudosclerotial plates, which appeared as black zone lines in cross section. In contrast, direct infections through unwounded root tissues could be recognized macroscopically by the presence of swellings and cracks in the bark; rhizomorphs were often associated with these root lesions. Further examination of the root lesions revealed the presence of mycelium in the phloem and brown stain in the adjacent xylem; *A. sinapina* and *A. ostoyae* could not be distinguished by their appearance in the phloem. Confirmation of *Armillaria* infections through wounded and unwounded root tissues was accomplished through isolation and identification.

The majority of *A. sinapina* infections occurred through wounds, whereas most *A. ostoyae* infections occurred in the absence of wounds (Table 2-2).

Table 2-2. Wounded and unwounded surface areas on parent roots, the number of *Armillaria* infections and the number of infections per m^2 of wounded and unwounded tissues, by cut block.

Cut Block	Surface Area [1] (cm^2)		# *Armillaria* Isolated [2]				# *Armillaria* Infections/m^2 [3]			
	W	UW	*A. ostoyae*		*A. sinapina*		*A. ostoyae*		*A. sinapina*	
			W	UW	W	UW	W	UW	W	UW
14	62	9 424	4	5	2	0	645	5.3	323	0
26	263	12 547	0	2	3	0	0	1.6	114	0
61	421	11 264	2	2	8	2	47	1.8	190	1.8
80	366	9 438	0	3	12	1	0	3.2	328	1.1
M14	80	9 365	6	8	1	0	750	8.5	125	0
M16	267	11 676	0	7	14	0	0	6.0	524	0
Total/ Average	1480	63 714	12	27	40	3	240	4.4	267	0.48

[1] Total wounded (W) and unwounded (UW) surface area of parent roots in each cut block.
[2] The number of *A. ostoyae* and *A. sinapina* isolates obtained from wounded (W) and unwounded (UW) tissues.
[3] The number of *A. ostoyae* and *A. sinapina* isolates obtained per m^2 of wounded (W) and unwounded (UW) tissues.

Ninety-three percent (40/43; Table 2-2) of *A. sinapina* infections were associated with wounds, while only 31 percent (12/39; Table 2-2) of *A. ostoyae* infections were associated with wounds. Considering the number of infections per m^2, both *A. sinapina* and *A. ostoyae* demonstrated an association with wounds. *Armillaria sinapina* was isolated approximately 600 (267/0.48; Table 2-2) times more often per unit area of wounded than unwounded tissue, whereas, *A. ostoyae* was isolated approximately 50 (240/4.4; Table 2-2) times more often per unit area of wounded than unwounded tissue. These results were reflected by the ANOVA, which indicated that averaged over both species, *Armillaria* infections were more common per unit area of wounded than unwounded tissue ($p=0.0012$). The relative number of *A. ostoyae* and *A. sinapina* infections per unit area of tissue varied between wounded and unwounded tissues, as indicated by the significant wound status by *Armillaria* species interaction ($p=0.0207$).

The lack of a site preparation technique effect (p=0.6581), or an interaction of site preparation technique with wound status (p=0.7974) and *Armillaria* species (p=0.6459) indicates that the results were independent of site preparation technique.

The probability of a wound being infected by *A. sinapina* varied with cut block (p=0.0036), but did not vary with either the type or size of the wound. The majority of the wounds were between 0.01 and 4.00 cm^2 in size (Figure 2-5).

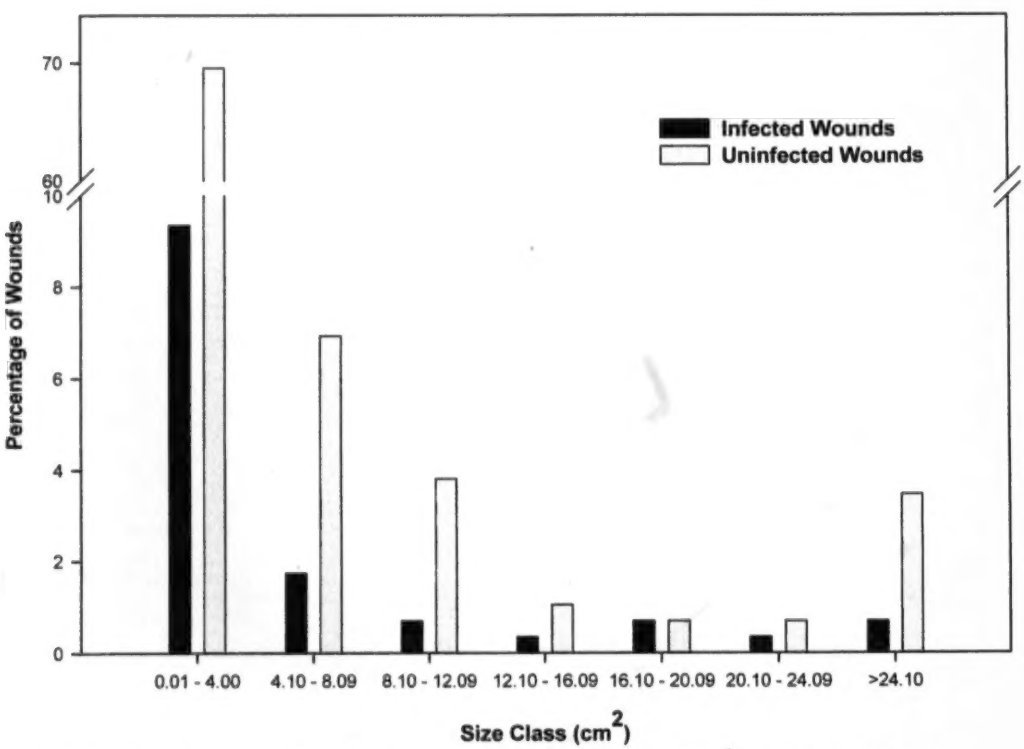

Figure 2-5. Percentage of *A. sinapina* wounds by size class (cm^2) and infection class (infected or uninfected) for 205 scrapes, 66 severs and 18 sever/scrape combinations. There were a total of 40 infected wounds.

There was no association between compartmentalization and wound type (p=0.7688), indicating that the probability of an *A. sinapina* infected wound being compartmentalized was similar for all wound types. On average 40 percent of the *A.*

sinapina infected wounds were compartmentalized. *Armillaria* infected wounds in which the stain and decay did not encompass the entire diameter of parent root present at the time of wounding may not necessarily have been compartmentalized since the pathogen may not have had enough time to grow into the tissues formed subsequent to wounding. However, since *A. sinapina* had failed to reach the location of wall 4 for only 5 percent of the infections, compartmentalization was not likely to have been greatly overestimated.

The four most commonly isolated Deuteromycotina were *Acremonium berkeleyanum* (Karsten) Gams, *Phialophora melinii* (Nannf.) Conant, *Phialophora bubakii* (Laxa) Schol-Schwarz, and *Phialophora alba* v. Beyma (Table 2-3).

Table 2-3.. Percentage of wounds by type that yielded at least one isolate of *Armillaria* or one of four commonly isolated Deuteromycotina. More than one microorganism may have been associated with the stain/decay of each wound.

Species	Scrape	Sever	Sever/Scrape
A. ostoyae	3.4	7.5	0.0
A. sinapiina	15.0	11.9	26.3
A. berkelleyanum	22.2	17.9	21.1
P. meliniii	32.9	28.4	31.6
P. bubakcii	9.2	7.5	15.8
P. alba	22.2	28.4	36.8

A large number of different fungi were isolated in small numbers and grouped in the category "'unknown fungi," the majority of which were Deuteromycotina (Table 2-4). A small number of unknown clamped fungi were also encountered (Table 2-4).

Table 2-4. Total number of wounds by type yielding unknown fungi, unknown clamped fungi and bacteria. More than one microorganism may have been associated with the stain/decay of each wound.

	Scrape	Sever	Sever/Scrape	Total
Unknown Fungi	114	43	13	170
Unknown Clamped Fungi	10	2	2	14
Bacteria	21	13	2	36

NOTE: Isolations from 25, 4, 1, 6 scrapes, severs, sever/scrapes and presumptive severs, respectively yielded no microorganisms.

2.4 Discussion

This study demonstrates important differences between *A. sinapina* and *A. ostoyae*. Whereas *A. sinapina* was more frequently isolated from wounds than *A. ostoyae* (Table 2-2), *A. ostoyae* was more likely to be associated with unwounded root tissues than *A. sinapina*. When the results were adjusted for surface area available, *A. ostoyae* was more common per unit area of wounded than unwounded tissue (Table 2-2); however, this 'preference' for wounds was more pronounced for *A. sinapina* than *A. ostoyae*. The extensive rhizomorph network of *A. sinapina* in soil and on root surfaces (Cruickshank *et al.* 1997) may increase the probability of encountering wounds; *A. ostoyae* produces fewer rhizomorphs than *A. sinapina* (Cruickshank *et al.* 1997). The greater occurrence of *A. ostoyae* on unwounded root tissues than *A. sinapina* suggests that *A. ostoyae* is more pathogenic on aspen than *A. sinapina*. These results are consistent with the findings of other researchers; Banik *et al.* (1995) determined that *A. ostoyae* was more pathogenic on aspen than *A. sinapina*. Morrison *et al.* (1985) noted that *A. sinapina* was weakly pathogenic as it was typically found on suppressed or overmature broadleaved tree species.

In analyzing the results of this study the possibility was considered that the potential for *Armillaria* infection through unwounded root tissues might have been

increased by the presence of nearby wounds. Unwounded root tissues may not have been exempt from stress resulting from wounding, as unwounded root areas were commonly interrupted by several wounds; within 30 cm of the stem a given root system could have had anywhere from one to six wounds. Root systems were cross classified according to the presence or absence of infections through unwounded tissues and the presence or absence of wounds on the same root system. A chi-square test to determine the association between infection of unwounded tissues and the occurrence of wounds provided no evidence ($p=0.7586$) that infections through unwounded root tissues were influenced by adjacent wounds.

Consistent compartmentalization of *A. sinapina* infected wounds as described in the CODIT (Compartmentalization of Decay In Trees) model (Shigo and Tippett 1981; Shigo 1984) was not observed, as only about 40 percent of the wounds were compartmentalized. This result is consistent with Stanosz and Patton (1987) who observed that stain and decay occurred throughout the entire root diameter of aspen. Robinson and Morrison (2001) noted that compartmentalization of *A. ostoyae* did not occur in the roots of 6 to 8 year old western larch [*Larix occidentalis* Nutt.] or Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*] trees. Compartmentalization may have been overestimated in approximately 5 percent of the *A. sinapina* infected wounds, as the associated stain and decay had not yet reached the boundary within which compartmentalization was determined to have occurred. Thus it is possible that had compartmentalization been assessed in cut blocks site prepared more than 10 years previously, the proportion of compartmentalized *A. sinapina* would have been slightly lower than that observed in the current study.

The ability of *Armillaria* to grow through the barrier zone produced by the cambium in response to wounding, which is wall 4 of the CODIT model (Shigo and Tippett 1981; Shigo 1984) could account for the lack of compartmentalization observed in 60 percent of the *A. sinapina* infected wounds. An alternative explanation to the CODIT model is that non-specific mechanisms rather than an active response by the tree may be responsible for maintaining sapwood function adjacent to damaged or non-functional tissue (Boddy and Rayner 1983). This alternative attributes the shape of the stain and decay patterns in trees to changes in water and aeration following wounding (Boddy and Rayner 1983). The xylem is brought into direct contact with dry air following woundig (Rayner 1986), resulting in a more favourable growth environment for many fungi. Wounding enables fungi unadapted to the growth conditions in an intact tree to colonize woody tissue (Boddy and Rayner 1983). Barrier and reaction zones arise through the formation of tyloses and/or deposition of gums, resins or suberised layers and by the accumulation of polyphenolic extractives (Rayner 1986). These zones form at sealant layers that limit the spread of cavitation and drying; therefore, they only indirectly contain the spread of fungi (Rayner 1986; Rayner and Boddy 1988).

Infection by *A. sinapina* may not require large changes in aeration within the sapwood following wounding. Oxygen controls the rate of growth and morphology of rhizomorphs (Smith and Griffin 1971). High rates of oxygen diffusion within the central canals of rhizomorphs are required for maximum growth; however, partial pressures in excess of 0.04 atm in contact with the outside surfaces of rhizomorphs inhibit growth (Smith and Griffin 1971). Consequently, rhizomorphs of *Armillaria* are capable of invading the anoxic conditions present in non-aerated sapwood (Rayner and Boddy

1988). The tolerance of *A. sinapina* to anoxic conditions could account for the large proportion of non-compartmentalized wounds observed in this study.

It was thought that large wounds would have a greater probability of infection by *A. sinapina*, due to the large surface area available for rhizomorphs to contact; *A. sinapina* produces an extensive rhizomorph network in soil and on root surfaces (Cruickshank *et al.* 1997). However, the results of the current study determined that the proportion of wounds infected by *A. sinapina* was not influenced either by wound size or wound type. The lack of a significant size effect suggests that large wounds were not required for infection by *A. sinapina*. The sample size of large wounds targeted by *A. sinapina* may have been too small to detect a size effect; the majority of the wounds were between 0.01 and 4.00 cm^2 (Figure 2-5). Futhermore, wound size was underestimated, as sampling was conducted 8 to 10 years following wounding; the vascular cambium would have initiated the formation of callus tissue.

Wounding of aspen parent roots was a frequent occurrence; there were only 28 unwounded root systems sampled in this study. The proportion of root wounds was probably underestimated, as small wounds may have had sufficient time to completely heal over. However, this underestimation would not affect the interpretation of the results, as there were no stain and/or decay fungi associated with these wounds. For example, the presumptive severs observed in this study were likely very small diameter parent roots, which healed over within the first growing season following wounding; microorganisms would not have had enough time to become established in these wounds. Small scrape wounds would also have had sufficient time to initiate callus tissue and heal over, preventing colonization by microorganisms.

The pattern of wounding did not change with the site preparation technique (ripper plow versus disk trencher). Sever wounds were associated with furrow locations; scrape wounds were located in both the furrow and interfurrow locations. The occurrence of scrape wounds in the interfurrow areas of the cut block was likely related to the timing of harvesting and site preparation. The majority of the cut blocks were site prepared between June and August (Table 2-1); tire traffic in the cut block during periods when the ground was not frozen could account for the occurrence of scrape wounds in the interfurrow locations. Forestry operations conducted during the winter, when the ground is presumably frozen and the bark is strongly attached to the sapwood, reduce the severity of damage to roots and stems (Vasiliauskas 2001).

Although all of the cut blocks were located within the e2 (low-bush cranberry AW) ecosite phase of the lower foothills subregion of Alberta, infection by *A. sinapina* varied with site ($p=0.0036$). The amount of woody debris remaining on a site following harvesting and site preparation operations increases the amount of substrate available to *Armillaria*. The density of aspen present on the site prior to harvesting could affect the amount of infection by *A. sinapina*, as infested stumps provide a foodbase for rhizomorph extension (Mallett 1992). Stanosz and Patton (1990) observed the spread of *Armillaria* from stumps into the parent root system; the potential exists for parent roots to serve as a means of entry into sucker stems. Environmental factors such as soil nutrients, drought, waterlogging, insect injury and frost may predispose trees to attack by *Armillaria* (Mallett 1992). The incidence of infection by *A. ostoyae* increased significantly with decreasing NH_4^+ concentration of the surface organic horizon (Mallett and Maynard 1998). Soil type affects inoculum viability, rhizomorph production, frequency of

seedling infection, and the likelihood of tree death following infection (Blenis *et al.* 1989). Heavier soils (clay loam) were found to be the least favourable for *A. ostoyae* and lighter soils (sandy loam) the most favourable (Blenis *et al.* 1989; Mallett and Maynard 1998). McLaughlin (2001) observed that *A. ostoyae* was found more frequently on rapidly drained sites than on well-drained or poorly drained sites; *A. sinapina* was usually not found on well-drained sites, but was more frequently found on poorly drained sites.

Pathogenic fungi other than *Armillaria* were not associated with root wounds of aspen in the current study. The age of the harvesting and site preparation wounds (8 to 10 years) sampled may account for the lack of non-*Armillaria* decay causing fungi. Basham (1958) observed a correlation between age and decay; older stands typically contained higher proportions of decayed heartwood than younger stands. The following non-*Armillaria* decay fungi were isolated at very low frequencies 10 years after scarification: *Coprinus micaceus*, *Coriolus zonatus*, *Flammulina velutipes*, *Pholiota spectabilis* and *Peniophora cinerea* (Basham 1988). These fungi do not appear to be important wound parasites of aspen in the cut blocks sampled in this study. The frequency of these fungi may increase as the cut blocks approach rotation age, since the number of infection courts and the time they have been available for infection increases with increasing age (Basham 1958).

Deuteromycotina fungi in the genus *Phialophora* were the most frequently isolated non-*Armillaria* fungi associated with root wounds; species included *P. melinii*, *P. bubakii* and *P. alba*. Basham (1988) observed that *P. alba* was frequently associated with scarification wounds. Ross (1976) reported that an unknown species of *Phialophora* appeared to be operating as an aggressive parasite of root wounds. Ascomycotina fungi

are not typically associated with the decay of wood (Basham 1988). However, hymenomycetes are commonly found growing through tissues already colonized by other organisms (Shigo 1967); bacteria and non-hymenomycetous fungi are believed to play a role in the detoxification of phenolic substances produced by trees in response to injury and infection (Rayner and Boddy 1988).

In conclusion, wounding of the parent roots of aspen was a frequent occurrence in cut blocks site prepared for the regeneration of white spruce. Both *A. sinapina* and *A. ostoyae* were wound pathogens of aspen; however, the two species differ biologically. *Armillaria sinapina* was more frequently associated with wounds than *A. ostoyae*. In contrast, *A. ostoyae* was better able to attack unwounded root tissues, indicating that it is more pathogenic on aspen than *A. sinapina*. Approximately 60 percent of *A. sinapina* infected wounds were non-compartmentalized, indicating that the potential exists for *Armillaria* to advance from the parent roots to the sucker stems. Differences in *A. sinapina* infection were attributed to site differences among the six cut blocks. Sever wounds were associated with furrow locations; scrape wounds were located in both the furrow and interfurrow locations. This relationship did not change with the site preparation technique (ripper plow versus disk trencher). Pathogenic fungi other than *Armillaria* were not observed on wounded roots, suggesting that non-*Armillaria* decay causing fungi were not wound parasites of aspen. The results indicate that site disturbances that injure roots increase the incidence of *A. sinapina* more than *A. ostoyae*.

2.5 Literature Cited

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Chapter 3

Field Inoculation of Aspen Roots with *Armillaria sinapina*

3.1 Introduction

Following site disturbances, aspen [*Populus tremuloides* Michx.] reproduces vegetatively by suckers from the shallow lateral roots (parent roots) of the previous stand (Schier 1973; Peterson and Peterson 1992). Wounds resulting from scarification performed for the regeneration of white spruce [*Picea glauca* (Moench) Voss] significantly increase the amount of stain and decay in the root systems and stems of aspen (Basham 1988).

Armillaria is an important forest pathogen of deciduous and coniferous trees (Wargo and Shaw 1985). *Armillaria mellea* (Vahl:Fries) Kummer was once thought to be the cause of the disease *Armillaria* root rot (Mallett 1992). It is now known that there are several reproductively isolated groups of *Armillaria* (Anderson and Ullrich 1979). In North America, these groups are termed North American biological species (NABS) (Mallett 1992); currently there are nine NABS (Dumas 1988; Mallett 1992). *Armillaria ostoyae* (Romagn.) Herink (NABS I) and *A. sinapina* Bérubé & Dessureault (NABS V) are the only two species of *Armillaria* known to occur in Alberta (Mallett 1990). *Armillaria ostoyae* is more pathogenic on aspen than *A. sinapina* (Banik *et al.*1995); however, *A. sinapina* is more commonly associated with hardwood species than *A. ostoyae* (Blodgett and Worrall 1992). The extensive rhizomorph network of *A. sinapina* enables it to exploit available food bases; *A. sinapina* produces greater amounts of rhizomorphs than *A. ostoyae* (Cruickshank *et al.* 1997). The objective of this study was to determine if *A. sinapina* is a wound pathogen of aspen.

3.2 Materials and Methods

3.2.1 Inoculum Preparation

The method used to prepare the inoculum was a modification of that described by Mallett and Hiratsuka (1988). Aspen stem segments (10 x 3cm) were placed upright in 1-L volume canning jars, four segments per jar. Approximately 200 mL of malt extract-dextrose broth (3% malt extract, 2% dextrose, 0.5% peptone, in distilled water) was added to each jar. Holes were punched in the jar lids, which were then covered with filter paper prior to closing. The jars were autoclaved at 121 C and 15 psi for 60 minutes, following which they were removed and left to stand overnight at room temperature. The malt extract-dextrose broth was then added to each jar to bring the volume up to approximately 200 mL; the jars were autoclaved for an additional 60 minutes.

A single isolate of *A. sinapina* obtained from an aspen root was grown on carrot agar (300 g carrots; 17 g agar; 800 mL distilled water) in the dark at room temperature for 5 weeks. The agar was cut into 1-cm^2 pieces and placed on the tops of the branch segments protruding from the malt extract-dextrose broth within the jars. The jar lids with the filter paper were then tightly closed. The inoculum blocks were incubated in the dark at room temperature for 4 months. Prior to transporting the inoculum to the field, the blocks were removed from the jars and placed on a bench to allow the fungal material to adhere to the surface of the inoculum blocks overnight.

3.2.2 Site Selection

A single field site was selected within the Weyerhaeuser Edson Forest Management Area, which had been site prepared using a ripper plow 10 years previously for the planting of white spruce seedlings. The density of white spruce and aspen in the

cut block was approximately 1700 and 4000 trees per hectare, respectively. The cut block was located within the e2 (low-bush cranberry AW) ecosite phase of the lower foothills subregion of Alberta and was characterized by a mesic moisture regime, medium nutrient regime and moderately fine to fine-textured till or glaciolacustrine parent matterials (Beckingham *et al.* 1996).

3.2.3 Inoculation

In early spring, 48 aspen trees were located along a transect across the field site; the trees were spaced at 10 metre intervals. A 30 cm segment of unwounded aspen root having a diameter between 1.0 and 2.5 cm was excavated adjacent to each tree. An inoculum block was bound to the root surface approximately 20 cm from the stem with plastic cable ties. For each odd numbered tree a wound (5 x 2 cm) was made on both sides and within 0.5 cm of the inoculum block; at even numbered trees the aspen root was left unwounded. The inoculated aspen root was covered with the soil and duff removed during the excavation process.

3.2.4 Retrieval and Analysis

Originally the inoculum was to be left in the field until early October and the dry weight of rhizomorphs of the wounded and unwounded roots compared using a t-test. However, upon retrieval of five of the inoculum blocks it was determined that rhizomorph growth had not occurred and some of the wounds had nearly completely callused over within one growing season. It was then decided to leave the inoculum in the field until the following September.

The remaining inoculum blocks were retrieved the following September. The aspen root segments to which the inoculum blocks were bound were removed using hand pruners; approximately 25 cm of aspen root was removed. Any rhizomorphs associated with the inoculum blocks were also collected.

In the laboratory, the aspen root segments were examined for any evidence of infection by *A. sinapina*, such as rhizomorph attachment on the root segment surface. A cross section was made through both wounded and unwounded root segments to look for evidence of stain and decay in the xylem. The wounds were examined to determine the extent to which they had callused over. Several of the inoculum blocks were sectioned longitudinally using a hand saw to determine the extent of colonization by *A. sinapina*.

3.3 Results

It was determined that the inoculation experiment was unsuccessful. Upon examination of the root segments in the laboratory it was determined that very few rhizomorphs were produced. Sectioning of the aspen roots revealed that infection by *A. sinapina* had not occurred. The majority of the wounds had nearly completely callused over. Stain was visible throughout the entire block of the sectioned inoculum blocks; however, there was no evidence of extensive decay in the wood.

3.4 Discussion

The results of this study indicate that more time may have been required to incubate the inoculum blocks in the lab for more extensive colonization by *A. sinapina* to occur. It is possible that a smaller inoculum block incubated for the same duration would have been more extensively decayed and resulted in a greater production of rhizomorphs.

Pieces of *Armillaria* infested wood in the soil may remain active sources of inoculum for decades (Mallett 1992). It appears that the resources in the inoculum blocks were not depleted within the two growing seasons over which the study occurred.

The precipitation levels throughout the duration of the study were considerably lower than other years, which could have resulted in dryer than normal soil conditions. McLaughlin (2001) observed that *A. sinapina* is more commonly associated with poorly drained sites. The low soil moisture content could possibly have affected the production of rhizomorphs in this study.

The nearly complete callus formation observed in the majority of the wounded root systems suggests that the wounds created in this study were not large enough. The annual increment of radial growth determines the rate of wound closure (Vasiliauskas 2001). The rate of wound closure may have increased in this study, as live aspen suckers were present within 20 cm of each wound. The wounds resulting from harvesting and site preparation operations typically occur prior to sucker initiation. As a result, there may have been more resources available to initiate callus formation in this study than there are in the absence of live suckers. In addition, the parent roots of aspen tend to be thicker on the distal side of suckers, as opposed to the proximal side (Brown 1935; Day 1944); indicating that there is a greater translocation of food in the distal portion of the root (Day 1944). Wounds located on the distal side of parent roots, may have had more resources available to initiate callus formation than those located on the proximal side.

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Chapter 4

Study Evaluation

4.1 Introduction

Aspen [*Populus tremuloides* Michx.] and white spruce [*Picea glauca* (Moench) A. Voss] mixtures are common throughout the boreal mixedwood forest of Canada (Lieffers *et al.* 1996). Root systems and stems of aspen injured by site preparation operations had significantly more stain and decay than uninjured suckers (Basham 1988). The current study indicates that both *Armillaria sinapina* Bérubé & Dessureault and *Armillaria ostoyae* (Romagn.) Herink are wound parasites of aspen. However, *A. sinapina* was more frequently associated with wounds than *A. ostoyae*. In contrast, *A. ostoyae* was more frequently associated with unwounded root tissues than *A. sinapina*, which suggests that *A. ostoyae* was more pathogenic on aspen than *A. sinapina*. Consistent compartmentalization of *A. sinapina* infected wounds was not observed 8 to 10 years following wounding; only approximately 40 percent of *A. sinapina* infected wounds were compartmentalized. Infection by *A. sinapina* varied with site differences among the six cut blocks. Non-*Armillaria* decay causing fungi were quite rare on parents roots wounded 8 to 10 years previously. This chapter will evaluate the study and discuss both the management implications of the results and the need for future research.

4.2 Study Evaluation

Although the inoculation experiment outlined in chapter 3 was deemed unsuccessful, the specific objectives of the entire research project were achieved. The results provide a greater understanding of the biology of *A. sinapina* and *A. ostoyae* on aspen, and the response of the parent root system to mechanical wounding. However, the sampling design restricts the application of the results. The current study has a limited ability to describe the effect of root wounding on: (i) individual clones; (ii) stands of fire

origin; (iii) the proportion of decay resulting from infection by *A. sinapina* at rotation age; (iv) the association of large wounds and infection by *A. sinapina*; and (v) the role of *Armillaria* in the next rotation.

Aspen is a clonal tree species that reproduces vegetatively by suckers (Schier 1973; Peterson and Peterson 1992). Barnes (1966) noted that aspen clones may be differentially susceptible to damage by biological agents, such as insects and fungi. The results of the current study do not indicate whether or not wounds of different clones respond similarly to infection by *Armillaria*, as either the individual tree or wound were the experimental unit. Clones in the northern mixedwood region are typically very small, normally a fraction of one hectare (Peterson and Peterson 1992); therefore, it is plausible that each root system in the current study represents an independent clone, as 30 root systems, spaced approximately 20 to 30 m apart, were collected from each cut block along a transect. A considerably larger sample size would have been required had the individual clone been the experimental unit, as sampling would have involved excavation of the parent root system of several sucker stems within each clone. Delineation of the boundary of individual clones would have been required prior to sampling. Individual clones could have been distinguished using aerial photographs combined with ground delineation using leaf, bark and stem characteristics (Kemperman and Barnes 1976).

Aspen suckering is stimulated by stand replacing disturbances, such as cutting or burning (Shier and Campbell 1978). Forest companies are interested in the comparison between fire origin stands and stands harvested and subsequently mechanically site prepared for the regeneration of aspen-white spruce mixtures. Basham (1993) noted that direct comparisons of the defectiveness of aspen stands of fire origin with those of

cutover origin may not be accomplished until sometime in the future, as prior to 1945 few stands originated from cutovers. A comparison of the amount of stem decay between the oldest cutover stands and fire origin stands of the same age could be made (Basham 1993). However, it would not be possible to determine the effect of mechanical root wounding at this age, as many wounds resulting from harvesting and site preparation operations in cut blocks older than 10 years would have had sufficient time to completely callus over. The sample size required to incorporate fire into a sampling design would have to be much larger; 8 to 10 year old fire origin stands would be required to make a comparison between the amount of stain and/or decay in the two types of stands. The number of wound categories would increase, as the type of wounds resulting from fire would be quite different from those resulting from site preparation. Cut block would be the experimental unit, as opposed to either the individual tree or wound; within each cut block many trees would need to be sampled. It is acknowledged that more information could have been gained had cut block been the experimental unit; however, the primary concern addressed by this study was the effect of mechanical root wounding on aspen quality.

The age of the cut blocks sampled does not provide an indication of the degree of compartmentalized *A. sinapina* infected wounds at rotation age. Many wounds categorized as compartmentalized may not have had enough time to overcome the boundaries within the tree that limit the spread of stain and decay. The possibility exists that more wounds may become infected by either *A. sinapina*, or some other decay causing hymenomycete prior to rotation age; Shigo (1967) noted that bacteria and non-hymenomycetous fungi often precede infection by hymenomycetes. Cut blocks site

prepared 8 to 10 years previously for the regeneration of aspen-white spruce mixtures were selected for sampling in order to provide enough time for fungal colonization of wounds, at the same time allowing for the identification of site preparation wounds. As a result, compartmentalization was probably slightly overestimated.

An assessment of compartmentalization of stain and/or decay in cut blocks site prepared 10 to 25 years previously for the regeneration of aspen-white spruce mixtures could provide a better indication of compartmentalization at rotation age. Such a study could build on the knowledge gained in the current study. However, identification of 10 to 25 year old site preparation wounds would be difficult and require more extensive sectioning of the parent roots, as few wounds could be identified macroscopically at this age.

Aspen suckers are heavily dependent on the parent root system for the first 25 years following suckering (Zahner and DeByle1965); live parent roots have been observed in mature aspen trees (Strong and LaRoi 1983). Sixty percent of all *A. sinapina* infected wounds in this study were non-compartmentalized; the potential exists for *Armillaria* to move from the parent roots to the root collar and secondary roots (Basham 1988). The suckering ability of aspen in the next rotation could be significantly reduced, should infection by *Armillaria* advance into the secondary roots, as suckering occurs on lateral roots with diameters between 0.5 and 2.5 cm (Peterson and Peterson 1992). As a result the role of *Armillaria* in the next rotation is largely unknown.

Wound size was not correlated with infection by *A. sinapina*, which suggests that large wounds were not required for infection. However, wound size at the time of sampling was not representative of wound size at the time of harvesting and site

preparation. *Armillaria sinapina* produces an extensive rhizomorph network in soil and on root surfaces (Cruickshank *et al.* 1997); therefore, one would expect that the probability of encountering wounds would increase with increasing wound size. The possibility exists that the sample size of large wounds was too small to detect a size effect, as the majority of the wounds were between 0.01 and 4.00 $cm.^2$ A controlled experiment could aid in the exploration of the relationship between *A. sinapina* infection and wound size. The results of chapter 3 indicate that the size of both the inoculum and the wound would be critical components of such a study; 10 cm^2 size wounds nearly completely callused over within one growing season.

4.3 Management Implications

The current study revealed that site preparation operations performed for the regeneration of white spruce cause extensive wounding to the parent root system of aspen. An effort should be made to reduce the amount of root wounding during harvesting and site preparation operations. The occurrence of scrape wounds in the interfurrow locations of the cut blocks may be the result of tire traffic during periods when the ground was not frozen or by the site preparation machine wheels or tracks. Damage to roots and stems may be reduced when forestry operations are conducted when the ground is frozen and the bark is strongly attached to the sapwood (Vasiliauskas 2001). Harvesting and/or site preparation operations in four of the cut blocks sampled in the current study were conducted between June and October, prior to the ground freezing. As a result, these cut blocks may have sustained more extensive wounding.

Root decay of aspen resulting from infection by *A. sinapina* may increase by rotation age, as 60 percent of *A. sinapina* infected wounds were non-compartmentalized

between 8 and 10 years following site preparation. Basal stem decay, reductions in height and diameter increment and tree mortality may result from extensive colonization of the root system by decay fungi such as *Armillaria* (Basham 1993). The increase in the amount of root system decay resulting from site preparation may also predispose scarified aspen to windthrow (Basham 1993). To minimize the loss of wood to stem decay, trees should be harvested at an age before excessive stem decay has developed (Basham 1993).

Planted white spruce seedlings may be more susceptible to infection by either *A. sinapina* or *A. ostoyae*. *Armillaria* may spread by either rhizomorphs produced from the roots of infected trees, or by root contact with infested wood material (Wargo and Shaw 1985). Aspen roots may provide a source of inoculum from which rhizomorphs may develop; the roots of white spruce seedlings may become infected upon contact with infected aspen roots. Infection of both aspen and white spruce regeneration could result in tree death; mixedwood stands may be understocked, which equates to a direct economic loss to forest companies, and changes in forest structure.

4.4 Future Research

The evaluation of the current study revealed that future related research is required. The boreal mixedwood region covers approximately 43.2 percent of Alberta's land area (Drew1988); therefore, knowledge of the regeneration of aspen-white spruce mixtures is essential. Forest managers need to understand the impact of harvesting and site preparation operations on the extent of aspen decay at rotation age, particularly by *A. sinapina*, in order to implement management strategies that reduce root wounding. Wounding of the parent root system of aspen may have created a previously unavailable niche for *A. sinapina*. Future research determining the effect of aspen root wounding and

Armillaria infection on: (i) different clones, (ii) stands of fire origin, (iii) the proportion of decay at rotation age, (iv) the association with large wounds, and (v) the next rotation will assist forest managers in successfully regenerating stands of aspen-white spruce mixtures.

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